LIST OF U.S. CUSTOMS LABORATORY METHODS

USCL NUMBER	METHOD	TITLE
34-01	ASTM D 1331 NHM - 1995	Test Methods for Surface and Interfacial Tension of Solutions of Surface-Active Agents
34-02	ASTM D 2357	Qualitative Classification of Surfactants by Infrared Absorption
34-03	USCL Manual	Organic Surface-Active Agents: Qualitative Analysis
34-04	ASTM D 2669 NHM - 1993	Test Method for Apparent Viscosity of Petroleum Waxes Compounded with Additives (Hot Melts)
34-05	ASTM D 3954 NHM - 1994	Test Method for Dropping Point of Waxes
34-06	ASTM D 2358	Test Method for Separation of Active Ingredient from Surfactants and Syndet Compositions
34-07	USCL Manual	Quantitation of Paraffin in Beeswax and Other Waxes by High Temperature Capillary Gas chromatography
34-08	USCL Manual	Quantitative Analysis of Paraffin in Beeswax by Column Chromatography

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-01 Index

ASTM D 1331
NHM - 1995
Test Methods for Surface and Interfacial Tension of Solutions of Surface-Active Agents

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

This method covers the determination of surface tension and interfacial tension of solutions of surface-active agents using either the du Nouy precision tensiometer or the du Nouy interfacial tensiometer.

The surface-active agent method is applicable to aqueous, nonaqueous, and mixed solvent solutions of surface-active agents. The interfacial tension method is applicable to two-phase solutions. More than one solute component may be present, including solute components that are not in themselves surface-active.

These methods are specifically applicable to commodities falling within the purview of HTSUS Heading 3402. However, they may also be useful in the analysis of organic mixtures containing surface-active agents which are found in Chapters 33 and 38.

2 REFERENCE

ASTM D 1331
NHM - 1995
Test Methods for Surface and Interfacial Tension of Solutions of Surface-Active Agents

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-02

Index

ASTM D 2357
Qualitative Classification of Surfactants by Infrared Absorption

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

This standard covers the qualitative classification of synthetic detergent products or mixtures of synthetic detergents. It is applicable to built detergent formulations as well as individual surfactant compositions.

Qualitative identification of surfactant type is based on the presence of infrared absorption bands attributable to specific functional groups. A listing of absorption bands corresponding to characteristic functional groups of some of the common types of surfactants are included in this method.

This method is applicable to soaps and organic surface-active products and preparations contained within Chapters 33, 34, and 38 of the Harmonized Tariff Schedule of the United States (HTSUS).

2 REFERENCE

ASTM D 2357
Qualitative Classification of Surfacts by Infrared Absorption

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-03 INDEX

Qualitative Analysis for Organic Surface-Active Agents

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

The following list of references contains procedures and references which should prove useful in the analysis of surface-active agents in different commodities. This list is being provided for general guidance and should not be considered exhaustive.

2 REFERENCES

Systematic Analysis of Surface-Active Agents, 2nd Edition
Rosen and Goldsmith
Wiley Interscience, NY, 1972

McCutcheon's Detergents and Emulsifiers, North American and International
McCutcheon's Division
MC Publishing Company
Ridgewood NJ, 1991

McCutcheon's Functional Materials
McCutcheon's Division
MC Publishing Company
Ridgewood NJ, 1991

Encyclopedia of Surfactants
Compiled by Michael and Irene Ash
Chemical Publishing, NY 1980

Formulary of Detergents and Other Cleaning Agents
Compiled by Michael and Irene Ash
Chemical Publishing, NY 1980

The Analysis of Detergents and Detergent Products
G.F. Longman
Wiley & Sons, NY 1975

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-04

Index

ASTM D 2669
NHM - 1993
Test Method for Apparent Viscosity of Petroleum Waxes Compounded with Additives (Hot Melts)

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

This method covers the determination of the apparent viscosity of petroleum waxes compounded with additives (hot melts) using a Brookfield Synchro-Lectric Viscometer. It applies to fluid hot melts having apparent viscosities up to 20 Pa · s at temperatures up to 175°C (347°F).

This test distinguishes between hot melts of different apparent viscosities, and is applicable to commodities falling within the purview of Headings 2712 and 3404 of the Harmonized Tariff Schedule of the United States (HTSUS).

2 REFERENCE

ASTM D 2669
NHM - 1993
Test Method for Apparent Viscosity of Petroleum Waxes Compounded with Additives (Hot Melts)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-05 Index

ASTM D 3954
NHM - 1994
Test Method for Dropping Point of Waxes

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

This method covers the determination of the ASTM dropping point for waxes using an appropriate dropping point apparatus.

This method is useful in determining the consistency of waxes, and is suitable for all types of waxes including, but not limited to, paraffin, microcrystalline, polyethylene, and natural waxes.

This method is applicable to commodities having waxy character falling within the purview of Headings 1521, 2712, 3404, and 3823 of the Harmonized Tariff Schedule of the United States (HTSUS).

2 REFERENCE

ASTM D 3954
NHM - 1994
Test Method for Dropping Point of Waxes

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-06

Index

ASTM D 2358
Test Method for Separation of Active Ingredient from Surfactantts and Syndet Compositions

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

This method covers the procedure for the separation and purification of the active ingredients from surfactant and synthetic detergent (Syndet) compositions. The separated active ingredients may be used for qualitative examinations. This method also permits the estimation of the total active ingredient level present in the sample.

The method involves the extraction of the active ingredient with alcohol. Reprecipitation of the insolubles is specified to remove the last traces of active ingredient. Dilution of the alcoholic extract to a known volume and the evaporation of a suitable aliquot permits measurement of total matter.

This method is applicable to organic surface-active products and preparations described in Chapters 33, 34, and 38 of the Harmonized Tariff Schedule of the United States (HTSUS).

2 REFERENCE

ASTM D 2358
Test Method for Separation of Active Ingredient from Surfactants and Syndet Compositions

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-07 Index

Quantitation of Paraffin in Beeswax and Other Waxes By High Temperature Capillary Gas Chromatography

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

0 INTRODUCTION

This method provides for the quantitation of paraffin-type hydrocarbons in beeswax and other waxes by high temperature capillary gas chromatography and for the subsequent determination of the amount of paraffin added to beeswax and other waxes. It utilizes a high temperature capillary gas chromatograph with an autosampler injecting a low concentration of sample onto a high temperature capillary column with a flame ionization detector to provide data for the quantitation of total paraffin- type hydrocarbons in beeswax and other waxes. This method provides adequate chromatographic resolution to clearly separate the even n-alkanes from the odd n-alkanes and most of the branched alkanes. An n-alkane internal standard is chosen such that it does not co-elute with any compounds normally present in the wax sample being analyzed. Formally, this is a standard additions gas chromatographic quantitative method with an internal standard.

While this method has many general uses, it has been developed to specifically provide for the quantitation of the total amount of paraffin of petroleum origin that has been added to a natural beeswax sample. The total paraffin-type hydrocarbons in each sample in a set of reference samples and the analysis sample is determined. The average of the total amount of paraffin-type hydrocarbons in the set of reference samples is then subtracted from that in the analysis sample to develop an assessment of the total amount of petroleum based paraffin that had been added to the analysis sample.

1 SCOPE

This capillary gas chromatographic standard additions method with internal standards is designed to quantitate the total paraffin-type hydrocarbons present in beeswax and other waxes. This method is applicable to paraffin-type hydrocarbons in waxes which span the n-alkane range from n-C20 through n-C39.

2 FIELD OF APPLICATION

This method is applicable to the determination of the total amount of paraffin-type hydrocarbons in a wax sample. These results can then be used to determine the total amount of paraffin wax added to articles of wax containing only beeswax and/or paraffin. The absolute accuracy of this method is dependent upon the reference

standards selected. The best references are the exact component beeswax and paraffin used in formulating the analysis sample. In their absence, the best obtainable representative standards must be used. This method can also be used to qualitatively detect the presence of waxes other than paraffin and beeswax in articles of wax by comparison with other reference chromatograms.

3 REFERENCES

This method does not reference any ISO methods.

4 DEFINITIONS

4.1 Beeswax

Beeswax is the wax of bees in the genus *Apis*, including the European bee *A. mellifera*, and the Asiatic species *A. dorsata, A. florea, and A. Indica.*

4.2 Laboratory sample

The laboratory sample is the sample as received in the laboratory for analysis by this method.

4.3 Analysis sample

The analysis sample is that portion of the laboratory sample that has been selected for preparation into a stock solution from which the test portion will be drawn and analyzed.

4.4 Reference sample

A reference sample is a laboratory reference sample that the laboratory analyzes by this method and whose results are used for comparison to the results of the analysis of the analysis sample by this method.

4.5 Paraffin

Paraffin is the hydrocarbon waxy portion of a petroleum crude oil that covers the n-alkane range from n-C20 through n-C39.

4.6 Paraffin-type hydrocarbons

Paraffin-type hydrocarbon is the hydrocarbon waxy portion of a wax that covers the n-alkane range from n-C20 through n-C39.

5 PRINCIPLE

This analysis method consists of the performance of each of the following steps in the listed order.

5.1 Selection of a representative portion of the analysis sample

5.2 Preparation of the analysis sample stock solution

5.3 Selection of a representative portion of each reference sample

5.4 Preparation of each reference sample stock solution

5.5 Preparation of the internal standard stock solution

5.6 Preparation of the standard additions analytical solutions

5.7 Gas Chromatographic analysis of the standard additions analytical solutions

5.8 Calculations

5.8.1 Calculation of the mass percent of paraffin-type hydrocarbons in the analysis sample

5.8.2 Calculation of the mass percent of paraffin-type hydrocarbons in each reference sample

5.8.3 Calculation of the mass percent of

added paraffin in the analysis sample

5.9 Report the results of analysis

6 REACTIONS

This method utilizes all chemical reactions associated with the equipment and technique of the analytical separation method of gas chromatography with flame ionization detection.

7 REAGENTS AND MATERIALS

7.1 General

7.2 Products used in their commercially available form

7.2.1 Toluene (T)

7.2.2 Chloroform

7.2.3 Beeswax, Paraffin, n-$C_{40}H_{82}$ (or other suitable alkane for an internal standard such as n-$C_{19}H_{40}$)

7.2.4 Compressed gasses:

Compressed gas cylinders of air and hydrogen of a purity suitable for high resolution gas chromatography. Air and hydrogen are used for the flame ionization detector (FID) and hydrogen is used for the carrier gas in the GC

7.3 Aqueous solutions

No aqueous solutions are used in this method.

7.4 Solutions with defined concentrations

7.4.1 Standard analysis sample volumetric solution

7.4.1.1 Definition:
Analysis Sample (AS)

This solution is the stock volumetric solution of the analysis sample in toluene prepared to a defined concentration.

7.4.1.2 Expression of concentration

The analysis sample shall be dissolved in toluene to a total volume of 100.0 mL. and a final concentration of 0.75 +/- 0.05 mg/mL.

7.4.2 Standard reference sample volumetric solution

7.4.2.1 Definition:
Reference Sample (RS)

This solution is the stock volumetric solution of the reference sample in toluene prepared to a defined concentration.

7.4.2.2 Expression of concentration

The reference sample shall be dissolved in toluene to a total volume of 100.0 mL. and a final concentration of 0.75 +/- 0.05 mg/mL.

7.4.3 Standard internal standard volumetric solution

7.4.3.1 Definition:
Internal Standard (IS)

This solution is the stock volumetric solution of the internal standard in toluene prepared to a defined concentration.

7.4.3.2 Expression of concentration

The internal standard shall be dissolved in toluene to a total

volume of 100.0 mL. and a final concentration of 0.50 +/- 0.05 mg/mL.

7.4.4 Standard Paraffin volumetric solution

7.4.4.1 Definition:
Paraffin Standard (PS)

This solution is the stock volumetric solution of paraffin in toluene prepared to a defined concentration.

7.4.4.2 Expression of concentration

The paraffin standard shall be dissolved in toluene to a total volume of 100.0 mL. and a final concentration of 0.75 +/- 0.05 mg/mL.

7.4.5 Standard standard additions volumetric solutions

7.4.5.1 Definition:
Standard Additions Standards (SAS)

These solutions are the analytical volumetric solutions for the complete set of standard additions with internal standards in toluene prepared to defined concentrations.

7.4.5.2 Expression of concentration:

Preparation of SAS vials

#	AS or RS µL	PS µL	IS µL	T µL	V µL
1	1000	0	350	400	1750
2	1000	100	350	300	1750
3	1000	200	350	200	1750
4	1000	300	350	100	1750
5	1000	400	350	0	1750

Final concentrations in the SAS vials

#	AS or RS mg/mL	PS mg/mL	IS mg/mL
1	0.4286	0.000	0.1000
2	0.4286	0.04286	0.1000
3	0.4286	0.08571	0.1000
4	0.4286	0.1286	0.1000
5	0.4286	0.1714	0.1000

7.4.6 Other solutions

There are no other solutions used in this method.

7.5 Indicators

No colorimetric indicators are used in this method.

7.6 Neutral alumina Brockman activity I or II

7.7 Glass wool

7.8 Heptane, spectro-grade

8 APPARATUS

8.1 Gas chromatograph and data system

H/P Model 5890 Series II gas chromatograph (GC), equivalent or better, with a HP 3396 integrator or Chemstation data system, equivalent or better, and an H/P 7673 autosampler, equivalent or better.

8.1.1 Compressed gas cylinders:

Air and hydrogen are used for the flame ionization detector (FID) and hydrogen is used for the carrier gas in the GC.

8.2 Capillary column:

12 meter SGE HT5 (5% phenyl polysiloxane-carborane) fused silica column, 0.25 mm inner diameter, 0.1

micron film thickness, equivalent or better

8.3 Volumetric flask: 100 mL, 50ml

8.4 Variable volume volumetric pipette: Eppendorf, equivalent or better, 100 uL and 1000 uL with tips

8.5 Analytical balance sensitive to 0.1 mg

8.6 Solution vials: glass, 20 mL scintillation counter vials with plastic screw closures

8.7 Autosampler vials: 2 mL clear glass GC autosampler vials with either screw or crimp closures

8.8 Water bath

8.9 Spatula

8.10 Knife

8.11 Coping saw

8.12 Any container capable of being sufficiently heated such that a wax sample placed within it will melt and whose volume is at least twice the volume of the quantity of the wax sample being melted.

8.13 Vortex Mixer

8.14 Disposable 10 mL serological glass pipette with top cut off at the 1 mL marking but below taper

9 SAMPLING AND SAMPLES

9.1 Definitions

9.1.1 Laboratory sample

The laboratory sample is the sample as received in the laboratory.

9.1.2 Test sample

The test sample is either the analysis sample stock solution or the reference sample stock solution.

9.1.3 Test portion

The test portion is that portion of either the analysis sample or the reference sample that is drawn for actual analysis.

9.2 Sampling

The laboratory sample and all laboratory reference samples are official samples obtained by one or more Customs Officers.

9.3 Preparation of the test, reference, and internal standard samples

Two preparations are provided for obtaining a test sample from a laboratory sample or a reference sample from a laboratory reference sample. Either may be used in the general case, however, if sample inhomogeneity is either encountered or suspected, procedure **9.3.1** "Preparation of the test sample by melting" shall be used.

9.3.1 Preparation of the test sample by melting

The entire laboratory sample or laboratory reference sample is melted in a container of at least twice the volumetric capacity of the sample. Upon complete melting the sample is stirred and an aliquot of approximately 20 mL. is poured into a glass 20 mL. vial.

9.3.2 Preparation of the test sample by slicing a portion and melting

The long axis of the laboratory sample or laboratory reference sample is determined and measured. The sample is cut perpendicular to the long axis in thirds with a knife or

saw. A slice 1 cm in length, cut perpendicular to the long axis, from the middle of each third is obtained. The three slices are placed in a container of at least twice their volume, melted in a water bath at a temperature of approximately 90 degrees C and stirred. An aliquot consisting of either the entire quantity, if it does not exceed 20 mL, or a representative and approximate 20 mL. portion, of the sample is poured into a glass 20 mL vial.

9.3.3 Preparation of the internal standard sample by melting

The entire internal standard sample is melted in a container of at least twice the volumetric capacity of the sample. Upon complete melting the internal standard sample is stirred and an aliquot of approximately 20 mL is poured into a glass 20 mL vial.

10 PROCEDURE

10.1 Safety precautions

Normal and established safety precautions shall be observed when handling the chemicals and working with the instrumentation specified in this method.

10.2 Stock solution preparations

10.2.1 Preparation of the analysis sample (AS) stock solution from the test sample

Melt the test sample in its' vial. When the test sample is completely molten, vortex it for ten seconds.

With the mass of the sample determined to 0.1 mg, transfer approximately 75 mg of the molten test sample from either section **9.3.1** or **9.3.2** into a 100 mL volumetric flask. Fill this flask approximately half-full with toluene. Warm the flask gently in a water bath until the wax dissolves, then, allow it to cool to room temperature and add additional toluene up to the mark.

If this section **10.2.1** analysis sample stock solution fails to qualify per the criteria listed in section **10.2.6.1.1.2**, then the analysis sample stock solution must be prepared by the procedure in section **10.2.6.1.1.3**.

10.2.2 Preparation of the reference sample (RS) stock solution from the reference sample

Melt the reference sample in its' vial. When the reference sample is completely molten, vortex it for ten seconds.

With the mass of the sample determined to 0.1 mg, transfer 75 mg of the molten reference sample from either section **9.3.1** or **9.3.2** into a 100 mL. volumetric flask. Fill this flask approximately half-full with toluene. Warm the flask gently in a water bath until the wax re-dissolves, then, allow it to cool to room temperature and add additional toluene up to the mark.

10.2.3 Preparation of the internal standard (IS) stock solution from the internal standard sample

With the mass of the sample determined to 0.1 mg, transfer approximately 50 mg of the internal standard, $C_{40}H_{82}$, into a 100 mL volumetric flask. Fill this flask approximately half-full with toluene. Warm the flask gently in a water bath until the sample re-dissolves, then, allow it to cool to room temperature and add additional toluene up to the

mark.

10.2.4 Preparation of the paraffin standard (PS) stock solution from the paraffin standard sample

Melt the paraffin standard sample in its' vial. When the paraffin standard sample is completely molten, vortex it for ten seconds.

With the mass of the sample determined to 0.1 mg, transfer 75 mg of the molten paraffin standard sample into a 100 mL. volumetric flask. Fill this flask approximately half-full with toluene. Warm the flask gently in a water bath until the sample re-dissolves, then, allow it to cool to room temperature and add additional toluene up to the mark.

10.2.5 Preparation of the test portion standard additions solutions (SAS) with internal standards

For each analysis sample, prepare the set of five GC autosampler vials using the stock solutions prepared in section **10.2** and the volumetric quantities from the table in section **7.4.5.2.**

10.2.6 Gas chromatographic procedure

10.2.6.1 General procedure

10.2.6.1.1 Qualifying procedure for the analysis sample.

10.2.6.1.1.1 Qualitative Gas Chromatography

Using the gas chromatograph, autosampler, data system, gasses, and columns, as described in section **8.1**, **7.2.4**, and **8.2**, run a 2.0 uL Injection of a solution containing 1.0 mL. of the analysis sample (AS) or reference sample (RS) stock solution as described in section **10.2.1**. and **10.2.2,** respectively**,** plus 750 uL of toluene, using the GC instrumental conditions as described in the following section **10.2.6.2.**

10.2.6.1.1.2 Qualification criteria

If the **10.2.6.1.1.1** qualifying chromatogram indicates that the paraffin-type hydrocarbons lie entirely within the n-alkane range C-20 through C-39 and there **are not** any other significant non-alkane/non-alkene compounds within this range (as would be indicated by the absence of a group of large peaks at ~ n-C40, n-C38 and n-C36 retention times with n-C36 peak the smallest of the three), this analysis or reference sample is then considered to lie within the scope of this method and is now considered to be a qualified analysis or referencence sample.

If the qualifying chromatogram in **10.2.6.1.1.1** indicates that the paraffin-type hydrocarbons lie entirely within the n-alkane range C-20 through C-39 and there **are** other significant non-alkane/non-alkene compounds within this range (as would be indicated by the presence of a group of large peaks at ~ n-C40, n-C38 and n-C36 retention times with n-C36 peak being the smallest of the three), then this analysis or reference sample must be cleaned-up by the procedure in **10.2.6.1.1.3** before it is can be considered to lie within the scope of this

method and become a qualified analysis or reference sample.

10.2.6.1.1.3 Sample clean-up procedure to obtain a qualified analysis sample (AS) or reference sample (RS) stock solution.

With the mass of the sample determined to 0.1 mg, quantitatively transfer approximately 37.5 mg of the molten test sample, dissolved in 3 mL of hot heptane (rinse container with two additonal aliquots of hot heptane ~1mL each) additional, onto a small liquid chromatographic column (see **8.14**) containing 2 gm of neutral alumina. **Note,** immediately prior to elution column should have ~ 5 mL of hot heptane passed through it without going to dryness. Elute with 10 mL of hot (greater than 90 degrees C) heptane into a 50 mL volumetric flask. Alkanes through n-C44 will elute under these conditions.

Fill the flask ~ 3/4 full with toluene. Warm the flask gently in a water bath until the wax dissolves, then, allow it to cool to room temperature and add additional toluene up to the mark and mix.

The contents of the 50 mL volumetric flask are the analysis sample (AS) or reference sample (RS) stock solution.

10.2.6.1.2 General procedure for a qualified sample

Using the gas chromatograph, autosampler, data system, gasses, and columns, as described in section **8.1**, **7.2.4**, and **8.2**, run the test portion standard additions solutions with internal standards samples that were prepared in GC autosampler vials as described in section **10.2.5**., using the GC instrumental conditions as described in the following section **10.2.6.2.**, and following the test sample run sequence as described in section **10.6.2.3**.

10.2.6.2 GC instrument operating parameters

The following instrument parameters provide excellent resolution with the GC, autosampler, integrator, and column listed in section **8.1**, **8.1.1**, and **8.2**. The use of other instruments, columns, and data systems will require that these parameters be modified.

10.2.6.2.1 Initial temperature:
80 degrees C

10.2.6.2.2 Initial time:
0.0 minutes

10.2.6.2.3 Oven ramp rate:
15 degrees C per minute

10.2.6.2.4 Final temperature:
360 degrees C

10.2.6.2.5 Final time: 5 minutes

10.2.6.2.6 Injection temperature:
330 degrees C

10.2.6.2.7 Detector temperature:
370 degrees C

10.2.6.2.8 Carrier gas: Hydrogen

10.2.6.2.9 Column head pressure:

20 psi, hold for 15 minutes, then increase at 0.5 psi/minute

10.2.6.2.10 Injection mode: Splitless

10.2.6.2.11 Purge time on: 1.00 minute

10.2.6.2.12 Purge time off: 0.01 minute

10.2.6.2.13 Injector: Autosampler

10.2.6.2.14 Injection volume: 2 µL

10.2.6.2.15 Injection liner: 4mm split/splitless with silanized glass wool in the center

10.2.6.2.16 First syringe rinse: Chloroform

10.2.6.2.17 # of first washes: 5

10.2.6.2.18 Second syringe rinse: Toluene

10.2.6.2.19 # of second washes: 5

10.2.6.2.20 Integrator time off: 0.0 minutes

10.2.6.2.21 Integrator time on: 4.0 minutes

10.2.6.2.22 Chart speed: 1.0 inch per minute

10.2.6.3 Test sample run sequences

10.2.6.3.1 Test sample duplicate run sequence

Autosampler Vial Position	Vial description
1	Toluene
2	SAS vial # 1
3	SAS vial # 2
4	SAS vial # 3
5	SAS vial # 4
6	SAS vial # 5
7	Toluene
8	SAS vial # 1
9	SAS vial # 2
10	SAS vial # 3
11	SAS vial # 4
12	SAS vial # 5
13	Toluene

10.2.6.3.2 Comprehensive test sample duplicate run sequence

Autosampler Vial Position	Vial description
1	Toluene
2	Toluene
3	SAS vial # 1
4	Toluene
5	SAS vial # 2
6	Toluene
7	SAS vial # 3
8	Toluene
9	SAS vial # 4
10	Toluene
11	SAS vial # 5
12	Toluene
13	SAS vial # 1
14	Toluene
15	SAS vial # 2
16	Toluene
17	SAS vial # 3
18	Toluene
19	SAS vial # 4
20	Toluene
21	SAS vial # 5
22	Toluene

10.3 Blank test

If any chromatographic peak beyond the retention time for the alkane n-C16 for a toluene sample from autosampler vial position # 1 or # 13 in **10.2.6.3.1**, Test sample duplicate run sequence, is greater in area than 0.1 % of the n-C40 internal standard peak area then either the sequence shall be re-run until this criterium is satisfied or **10.2.6.3.2**,

Comprehensive test sample duplicate run sequence, shall be used.

10.4 Check test

The chromatographic system is considered to be adequate if baseline resolution is obtained with the operating parameters specified in **10.2.6.2**, GC instrument operating parameters, for the n-alkane n-C39 of a petroleum paraffin wax and the internal standard n-C40.

10.5 Matching test

If chromatographic carryover occurs from SAS vial # 1 into the chromatogram for SAS vial # 2 using **10.2.3.6.1**,Test sample duplicate run sequence, then **10.2.3.6.2**, Comprehensive test sample duplicate run sequence, shall be used.

10.6 Calibration

The chromatographic resolution of the system is considered to be adequate if baseline resolution is obtained with the operating parameters specified in **10.2.6.2**, GC instrument operating parameters, for the n-alkane n-C39 of a petroleum paraffin wax and the internal standard n-C40.

11 EXPRESSION OF RESULTS

11.1 Method of calculation

11.1.1 Percent paraffin-type hydrocarbons

$$y = \frac{\text{GC Peak areas from n-C20 through n-C39}}{\text{GC Peak area of the internal standard, n-C40}}$$

X = % petroleum paraffin added to the sample

An equation from the linear plot of the above x and y values obtained from the analysis of the prepared standard additions standards is obtained. This equation is then solved for y = 0. The absolute value of the intercept at y = 0 is the percent paraffin-type hydrocarbon present in the sample.

11.1.2 Petroleum paraffin added to wax

In the formulation of wax candles and other wax articles, petroleum paraffin is frequently added to the base wax, for example beeswax, for engineering purposes. This procedure provides a method to estimate the amount of this added paraffin.

To obtain an estimate of the amount of added petroleum paraffin contained in a laboratory sample with respect to a normal reference sample, it is necessary to subtract the amount of paraffin-type hydrocarbons in a suitable reference sample from the amount of paraffin-type hydrocarbons in the laboratory sample as determined by the results of the calculation in **11.1.1**. Two procedures are provided: the first, **11.1.2.1**, uses a measured value as determined by the results of the calculation in **11.1.1**, for a suitable reference sample or set of reference samples; the second, **11.1.2.2**, uses a literature or other accepted reference value for the amount of naturally occurring paraffin-type hydrocarbons in the reference sample.

11.1.2.1 Reference sample value procedure utilizing the results of the calculation in **11.1.1** to obtain an estimate of the percent of naturally occurring hydrocarbon in a laboratory

sample.

An estimate of the amount of paraffin that has been previously added to a laboratory sample is obtained by subtracting the amount of paraffin-type hydrocarbons in the laboratory reference sample from the amount of paraffin- type hydrocarbons in the laboratory sample where both measured amounts have been determined by the results of the calculation in **11.1.1**.

$$\% HC_{added} = (\%HC - \%HC_{rv}) / (1-(\%HC_{rv}/100))$$

where:

$\%HC_{added}$ = percent C20-C39 paraffin

%HC = C20-C39 paraffin-type hydrocarbon from **11.1.1**

$\%Hc_{rv}$ = % C20-C39 paraffin-type hydrocarbon in a reference standard

11.1.2.2 Literature or other reference value procedure to obtain an estimate of the percent of naturally occurring hydrocarbon in a laboratory sample.

An estimate of the amount of paraffin that has been previously added to a laboratory sample is obtained by subtracting the amount of paraffin-type hydrocarbons in an authentic reference sample as determined by a literature or other reference value from the measured amount of paraffin-type hydrocarbons in the laboratory sample as determined by the results of the calculation in **11.1.1**.

$$\% HC_{added} = (\%HC - \%HC_{lv}) / (1-(\%HC_{lv} / 100))$$

where:

$\% HC_{added}$ = percent C20-C39 paraffin

% HC = C20-C39 paraffin-type hydrocarbon from **11.1.1**

$\% Hc_{lv}$ = % C20-C39 paraffin-type hydrocarbon in a literature or other reference value

11.2 Precision

A set of data is considered to be valid if the precision specifications in **11.2.1** and **11.2.2,** and **11.2.3** are satisfied.

11.2.1 Retention time precision

Any two samples run in the same sequence shall have retention times within 0.05 min for the internal standard, n-C40.

11.2.2 Correlation coefficient precision

The correlation coefficient of the linear plot in **11.1** shall be greater than R = 0.995.

11.2.3 A set of data is considered to be valid if duplicate injections from the same test portion have internal standard corrected signal responses that are within 5 % of each other.

11.3 Accuracy

11.3.1 A set of data is considered to be accurate if all three of the precision statements in **11.2** are satisfied for the set of data and the result obtained from the analysis of a paraffin standard, since maintenance was last performed on the GC, is within +\- 5 % of the certified value for that standard.

12 SPECIAL CASES

This method is not applicable to any special cases outside the scope of this method.

13 NOTES ON THE PROCEDURE

13.1 Literature values of naturally occurring hydrocarbons in beeswax vary. One range for yellow beeswax, reported in Industrial Waxes Volume I, by H. Bennett is 10.4% to 13.6%. This method provided a value for a standard white beeswax of 13.7%.

13.2 The values obtained from this method are dependent on the standards used. Paraffin added to beeswax often has $C_{27}H_{56}$ as the predominant hydrocarbon. It can also be a lower or higher hydrocarbon. This method accounts for these differences.

13.3 If the paraffin peaks overlap the $C_{40}H_{82}$ peak, which elutes after the naturally occurring n-alkanes, but before esters, then a different internal standard must be used.

13.4 For the beeswax candles used in the development of this method, it was observed that their observed inhomogeneity was approximately 10 % RSD, relative standard deviation. When the same test portion of a beeswax candle sample was analyzed it was observed that the range of obtained results was approximately 1 % RSD. We thus note that considerable importance must be directed toward efforts to obtain representative test and reference samples from the supplied laboratory sample and laboratory reference sample. The two preparation procedures described in section **9.3.1** and **9.3.2** are provided to assist in these efforts.

13.5 This method is applicable to the analysis of paraffin-type hydrocarbons in waxes which span the n-alkane range from n-C20 through n-C39. Literature values and our experience indicate that the range of paraffin-type hydrocarbons typically found in beeswax are primarily the odd n-alkanes from n-C23 through n-C35 with a small amount of even n-alkanes and n-alkenes, although exceptions may exist.

14 TEST REPORT

The test report for a laboratory sample, shall report, at a minimum, the percent paraffin-type hydrocarbons present in the test portion of the laboratory sample. If the test report also reports the percent added paraffin in a laboratory sample the test report shall also report both the percent paraffin-type hydrocarbons present in the laboratory sample and the percent paraffin-type hydrocarbons present in the reference (for example, beeswax) reference sample.

15 SCHEMATIC REPRESENTATION OF PROCEDURE

A schematic representation of the procedure is not applicable to this method.

16 BIBLIOGRAPHY

16.1 Kolattukudy, P. E., "Chemistry and Biochemistry of Natural Waxes", Elsevier, 1976, ISBN 0-444-41470-3.

16.2 Bennett, H., “Industrial Waxes”, Chemical Publishing Company, New York, 1975.

16.3 Tulloch, A. P., “Beeswax - composition and analysis”, Bee World, 1980, 61, 47-62.

16.4 Downing, D. T., Australian J. Chem., **14**, 253 (1961).

17 ANNEXES

This method does not contain any additional annex reference material.

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 34-08

Index

Quantitative Analysis of Paraffin in Beeswax By Column Chromatography

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

0 INTRODUCTION

This method provides for the quantitative measurement of paraffin-type hydrocarbons in beeswax and beeswax-paraffin mixtures by column chromatography and subsequent

determination of the amount of paraffin added to beeswax. The method consists of three procedures. First, the presence of paraffin-type hydrocarbons in a sample is identified by a qualitative screening analysis using either gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS). Second, for samples determined to contain paraffin-type hydrocarbons, the paraffin-type hydrocarbons are separated quantitatively by column (liquid-solid) chromatography. The percent weight of paraffin-type hydrocarbons in the sample is determined gravimetrically from the weight of solid percolate obtained from the eluent of the column. Third, the solid percolate collected in the quantitative analysis is

screened by GC, GC/MS, or Fourier transform infrared spectroscopy (FTIR) to verify that the percolate consists solely of paraffin-type hydrocarbons.

1 SCOPE AND FIELD OF APPLICATION

This method applies to beeswax, in the form of candles or in other forms, containing added paraffin. Specifically, the method has been formulated for quantitative measurement of paraffin-type hydrocarbons in candles composed of beeswax to which has been added an unknown quantity of paraffin. The method may be extended to quantitative analysis of paraffin-type hydrocarbons in other wax mixtures containing only two wax components, one of which is paraffin. The absolute accuracy of this method is dependent upon the reference standards selected, of which the best standards are the exact beeswax and paraffin used to manufacture the sample. If these materials are not available, the best obtainable reference standards must be used.

2 REFERENCES

ASTM D 1342
Test Method for Paraffin-Type Hydrocarbons in Carnauba Wax.

3 DEFINITION OF TERMS

3.1 Beeswax

Beeswax is the wax of bees in the genus Apis, including the European bee A. mellifera, and the Asiatic species A. dorsata, A. florea, and A. Indica.

Although the composition can vary in detail, beeswax primarily is composed of free wax acids and the esters of wax acids, with a lesser quantity of hydrocarbons. Typically, the hydrocarbon portion of beeswax consists of the odd chain length n-alkanes from $C_{23}H_{48}$ through $C_{35}H_{72}$, with a small amount of even chain length n-alkanes and alkenes.

3.2 Paraffin

Paraffin is the hydrocarbon waxy portion of a petroleum crude oil that covers the n-alkane range from approximately $C_{20}H_{42}$ through $C_{39}H_{80}$.

Typically, paraffin consists of a distribution of n-alkanes following the generic formula C_nH_{2n+2} (n is an integer), with smaller amounts of isoalkanes and cycloalkanes.

3.3 Paraffin-type Hydrocarbon

Paraffin-type hydrocarbon is the hydrocarbon waxy portion of a wax or wax mixture that covers the n-alkane range from $C_{20}H_{42}$ through $C_{39}H_{80}$.

3.4 Column

For the quantitative measurement of paraffin-type hydrocarbons (**5.2**), the column is defined to be the 250 mL cylindrical separatory

funnel packed with activated alumina.

3.5 Eluent

Eluent is the liquid mobile phase containing solvent plus dissolved sample collected from the end of the column.

3.6 Solid Percolate or Percolate

Solid percolate is the waxy residue remaining after evaporation of the solvent from the eluent collected from the column.

4 REAGENTS AND APPARATUS

Unless otherwise stated, all reagents are of analytical grade or better. All components of the apparatus are to be of condition equivalent to the stated condition or better, or having a sensitivity, precision and accuracy equivalent to or better than the stated components. Appropriate substitutions may be made provided these criteria are met.

4.1 *Reagents and Apparatus for Column Chromatography*

4.1.1 n-Heptane, 98.0% minimum by GC.

4.1.2 Activated Alumina (Al_2O_3), neutral or acidic, grade Brockmann activity I or II, or equivalent, Supelco #19,997-4, #19,996-6. Alumina should be stored at 100-110°C to maintain activity grade.

4.1.3 Refined Yellow Beeswax, m.p. 62-65°C, CAS No. 8012-89-3, Aldrich Chemical Co. #24,324-8.

4.1.4 Paraffin Wax, m.p. 52-58°C, CAS No. 8002-74-2, Aldrich Chemical Co. #31,765-9.

4.1.5 Cylindrical Separatory Funnel, 250 mL capacity, graduated, PTFE stopcock, VWR Scientific Products #30362-328.

4.1.6 Analytical Balance. Sensitivity of 0.1 mg is required for weighing solid percolate recovered from the eluent.

4.1.7 Drying Oven, capable of maintaining 100 +/- 5°C temperature.

4.1.8 Hot Plate.

4.1.9 Vibrating Mixer (Touch Mixer), Fisher Scientific.

4.1.10 Thermometer, 0-100°C.

4.1.11 For dissolving wax and evaporating solvent in ASTM D 1342: tall-form glass beakers, 600 mL to 1 L volume; glass beakers, 50 mL and 200 mL.

4.1.12 For melting wax samples to homogenize: glass jars, 100 mL to 250 mL or larger, and disposable aluminum pans, 100 mL to 200 mL or larger.

4.2 *Reagents and Apparatus for Gas Chromatography or Gas Chromatography/Mass Spectrometry*

4.2.1 Chloroform, 99.8+% minimum by GC.

4.2.2 Glass bottles, 4 oz., for storage of dissolved wax solutions (100 mL).

4.2.3 HP Model 5890 gas chromatograph (GC), equipped with either a flame ionization detector (FID) or a HP Model 5970 mass selective detector (MSD), or the equivalent or better.

4.2.4 Helium carrier gas (99.999%); hydrogen and compressed air detector gases for GC-FID.

4.2.5 25 meter HP-1 (crosslinked methyl siloxane) or HP-5 poly(5% diphenyl -

95% dimethylsiloxane) fused silica capillary column, 0.2 mm i.d., 0.33 micron film thickness for GC/MS, or the equivalent or better. A capillary column suitable for use at temperatures greater than 320°C may be substituted, such as aluminum or polyimide clad 12 m or 25 m SGE HT-5 (5% phenyl siloxane-carborane) or equivalent.

5 SAMPLE PREPARATION

Unless specified otherwise, wax from the sample candle, or other article or form, and reference beeswax and paraffin are to be prepared in the same manner.

5.1 *Homogenization of Sample and Reference Materials*

The sample wax or reference beeswax or paraffin may not be homogeneous in composition. To ensure a homogeneous composition is obtained, two approaches are recommended. In the first approach, the entire wax sample is melted to homogenize. In the second approach, approximately 20 to 40 g of wax is taken from several locations (top, bottom, interior) and melted. The choice of approach may depend on the size of the sample.

Melting is accomplished in a glass jar or beaker of appropriate size (twice the volume of the solid material) in an oven or water bath at approximately 90°C. The melt is swirled or stirred and poured into a preheated (90°C) aluminum pan (100 to 200 mL size for 20 to 40 g wax) and allowed to cool. A larger pan may be needed depending on the amount of sample or reference melted.

5.2 *Preparation for Qualitative Screening Analysis by GC or GC/MS*

Dissolve 1 g of the homogenized solid wax from the sample or reference from **5.1** in 30 to 50 mL warm chloroform or equivalent solvent in a 100 mL volumetric flask. After cooling, dilute to 100 mL and store in a 4 oz. glass bottle, or equivalent.

5.3 *Preparation for Quantitative Analysis by Column Chromatography*

Unless noted in this section, sample preparation follows the procedure specified in **ASTM D 1342** without modification.

Dissolve 2.0 g of the homogenized solid wax from the sample or reference from **5.1**, weighed to the nearest 0.01 g, in approximately 300 mL of boiling heptane in a 600 mL or 1 L tall-form beaker. The solution is maintained near the boiling point of heptane while it is added to the column in **6.2.3**.

6 PROCEDURE

Three procedures are specified. First, the presence of paraffin-type hydrocarbons is identified by a qualitative screening analysis using either GC or GC/MS. Second, the paraffin-type hydrocarbons are separated quantitatively by column chromatography and the weight recorded. Third, the solid percolate collected in the quantitative analysis is screened by GC, GC/MS, or FTIR to verify the presence of only paraffin-type hydrocarbons.

6.1 *Qualitative Screening Analysis by GC or GC/MS*

6.1.1 A solvent blank (1 microliter chloroform) is injected prior to the

sample or reference, under the conditions specified in **6.1.3**.

6.1.2 If undissolved wax solids are present it may be necessary to warm the bottle containing the sample or reference prior to injection. The glass bottle is warmed with a heat gun, or an aliquot placed in a sealed glass vial and held in a heating block. All of the wax solids should be dissolved and a clear (colored or colorless) solution obtained prior to injection.

6.1.3 1 microliter of the dissolved wax solution (sample or reference) is injected into the GC. The operating parameters of the GC and detector are those that are required to provide adequate resolution for determining the presence of paraffin-type hydrocarbons in the sample. These parameters are dependent on the specific capillary column and detector employed. For the HP-1or HP-5 column specified in **4.2.5** and an MSD detector, the following conditions are adequate:

Detector Temperature: 300°C
Injector Temperature: 290°C
Column Temperature:
hold 3 min at 100°C,
heat at 25°C/min to 200°C,
heat at 10°C/min to 320°C,
hold 20 min at 320°C.
Solvent Delay: 3 min
Injection: split or splitless
MSD: scan 40-600 m/z

6.1.4 A solvent blank (1 microliter chloroform) is injected after the sample or reference, under the conditions specified in **6.1.3**.

6.1.5 The presence of paraffin-type hydrocarbons in the sample is identified by comparison of the chromatograms (GC) or total ion chromatograms (GC/MS) obtained for the sample and reference beeswax and paraffin.

6.2 ***Quantitative Analysis by Column Chromatography***

Unless noted in this section, quantitative analysis follows the procedure specified in **ASTM D 1342** without modification.

The procedure utilizes a large volume of heptane, a flammable solvent, maintained at temperature close to the boiling point. Caution should be exercised in handling this solvent.

6.2.1 To assist the even packing of the alumina, the stem of the cylindrical separatory funnel is placed on a vibrating mixer for approximately 30 to 60 sec after tapping the side of the funnel sharply several times with the palm of the hand.

6.2.2 The column is warmed with sufficient volume of heptane at its boiling point such that the temperature of the eluent issuing from the funnel stem is at 50 to 55°C prior to addition of sample or reference solutions. The column should be tapped again as it is warmed.

6.2.3 The sample or reference prepared from **5.3** is introduced to the column by pouring as specified in **ASTM D 1342.**

6.2.4 After the last of the sample or reference solution has been added to the column, rinse the tall-form beaker with at least three (3) successive 20-mL minimum portions of fresh, boiling heptane and add the washings to the column.

6.2.5 1 or 2 portions of the eluent are tested by collecting approximately 20 mL in a 50 mL glass beaker and evaporating the solvent. Elution is

complete when only a trace of greasy residue is obtained in the beaker. Any residue or solid is added back to the percolate with hot heptane.

6.2.6 The saponification number of the percolate is not determined. The quantitative analysis procedure for a sample or reference is ended after the weight of paraffin-type hydrocarbons is recorded.

6.2.7 A known reference beeswax material is analyzed each time the column is prepared.

6.2.8 As specified in **ASTM D 1342**, attention must be given to ensure that no wax acids or esters are eluted and that no hydrocarbon is retained between samples.

6.3 ***Qualitative Screening Analysis of Percolate from Column Chromatography by GC, GC/MS or FTIR***

After the weight is recorded, the solid percolate collected from the eluent is screened by GC, GC/MS, or by FTIR to ensure that only paraffin-type hydrocarbons are present in the solid percolate.

6.3.1 For analysis by GC or GC/MS, the solid percolate is dissolved in chloroform in the proportion 0.01 g per mL solvent and analyzed as described in **6.1.3**. The chromatogram obtained by GC or total ion chromatogram obtained by GC/MS from the solid percolate is examined to verify that only paraffin-type hydrocarbons are present

6.3.2 For analysis by FTIR, a portion of the solid percolate is melted on a KBr plate under a heat lamp or in an oven. A thin film is formed by spreading the melt evenly across the plate with a glass micropipette or similar implement. The FTIR spectrum is recorded in transmission mode over the range 4000-600 cm^{-1}. The sample spectrum is then compared against spectra for reference beeswax and paraffin prepared and collected under the same conditions. Absence of a C=O peak (circa 1730 - 1740 cm^{-1}) in the spectrum of the solid percolate collected from the sample indicates that all of the wax acids and wax acid esters were retained on the column.

7 EXPRESSION OF RESULTS

The percent weight of paraffin in the sample is obtained from the measured percent weight of paraffin-type hydrocarbons in the sample, and from either the measured or known (literature) percent weight of paraffin-type hydrocarbons in reference beeswax and paraffin. The percent weight of paraffin in the sample is calculated from:

$$\%\ Paraffin = 100 * \frac{wHC_{sample} - wHC_{ref,\ bee}}{wHC_{ref,\ par} - wHC_{ref,\ bee}}$$

where in the equation:

wHC_{sample} = measured weight fraction of paraffin-type hydrocarbon in sample from **6.2** (g solid recovered from the eluent / g sample).

$wHC_{ref,\ bee}$ = measured weight fraction of paraffin-type hydrocarbon in reference beeswax from **6.2** (g solid recovered from the eluent / g beeswax), or from literature or

other reference value.

$wHC_{ref, par}$ = weight fraction of hydrocarbon in reference paraffin (g hydrocarbon / g paraffin). $wHC_{ref, par} = 1.0$

8 PRECISION

For the quantitative measurement of percent weight paraffin-type hydrocarbon in beeswax or beeswax-paraffin mixtures, the standard deviation obtained from replicate measurements should be less than 2%.

9 BIBLIOGRAPHY

9.1 Annual Book of ASTM Standards, Volume 15.04 Soap; Polishes; Leather; Resilient Floor Coverings, 1996, American Society for Testing and Materials, PA.

9.2 Encyclopedia of Chemical Technology, 3rd Edition, Volume 24, 1984, Wiley-Interscience, NY.

9.3 Industrial Waxes, Volume I, Natural and Synthetic Waxes, H. Bennett (Ed.), 1975, Chemical Publishing Co., NY.

10 NOTES ON THE PROCEDURE

10.1 In practice, a maximum of four analyses may be performed on a single column, one reference beeswax and three samples. In this regard, it is convenient to prepare two columns at a time to maximize the number of samples that may be analyzed in one session. If more than one sample is analyzed on a single column however, cautions regarding elution of wax acids and esters or retention of hydrocarbons noted in **6.2.8** and **6.3** must be heeded.

10.2 The percent weight of paraffin-type hydrocarbons in Refined Yellow Beeswax, CAS No. 8012-89-3, Aldrich #24,324-8, measured using this method yielded 14.7 +/- 0.4 % based on a limited number of analyses. However, the percent weight of paraffin-type hydrocarbons in this material has not been certified.